The potential of cell cultures for the production of salt tolerant cultivars

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Summary

The progress towards the production of salt tolerant plants through selection in cell cultures is briefly reviewed. The need for a fuller understanding of the mechanisms of salt tolerance in non-halophytes is emphasised and illustrated with reference to recent investigations on the role of proline. A clear protective effect, against salt stress, of exogenously applied proline has been demonstrated suggesting elevated levels of endogenous proline synthesis, for which there is a direct selection procedure, as a basis for improved salt tolerance.

Salt tolerance of proline-overproducing plants and cell cultures is currently under investigation in a *Nicotiana sylvestris* line with almost 100-fold increase in free proline.

Descriptors: selection, cell culture, salt tolerance, *Nicotiana sylvestris*, proline, hydroxyproline resistance

Introduction

For several reasons progress in the selection for salt tolerance in tissue and cell cultures seems a particularly appropriate topic to review within the context of this session. Firstly, improved salt tolerance is clearly a desirable agronomic trait. Secondly, it is one which has attracted considerable attention from a number of investigators over the last few years, with remarkably little success. It therefore serves to illustrate some of the difficulties already mentioned (King, 1984) in applying this conceptually simple approach to a practical problem. Finally, salt tolerance is an area in which recent physiological investigations, with both whole plants and cell cultures, have yielded insights which suggest new approaches to the selection of tolerant cultivars.

In the last ten years salt tolerant cell lines have been reported for:

- *Nicotiana sylvestris* (Zenk, 1974; Dix & Street, 1975)
- *Nicotiana tabacum* (Nabors et al., 1975; Hasegawa et al., 1980)
- *Capsicum annuum* (Dix & Street, 1975)
- *Medicago sativa* (Croughan et al., 1978)
- *Kickxia ramosissima* (Mathur et al., 1980)
- *Citrus sinensis* (Ben-Hayyim & Kochba, 1982)
- *Oryza sativa* (Yano et al., 1982)
- *Colocasia esculenta* (Nyman et al., 1983)

Only in the case of *N. tabacum* (Nabors et al., 1980) has sexual transmission of salt

tolerance, at the whole plant level, been satisfactorily demonstrated. This success may have provided encouragement and an added stimulus to other workers in the field. The problems with the other systems have been at the level of plant regeneration, transfer of plants to soil, expression of resistance in the whole plant, induction of flowering, fertility, or heritability. Sexual transmission of resistance in some lines may be currently under investigation. Both Yano et al. (1982) and Dix et al. (1983) report a reduced level of resistance in progeny compared to their parents, and in the latter case this is not found in intact seedlings, but only in callus derived from them.

There is a growing conviction that adaptation to salt stress may be a common feature in cell cultures, giving rise to epigenetic variants of sufficient stability to obscure the lower frequency occurrence of genuine genetic variants. This suggests that a careful reappraisal of the whole approach to the selection of salt resistant lines may be in order.

Cell cultures have also been used in investigations on the basis of salt stress damage and tolerance. Differences in salinity tolerance at the whole plant level are reflected by differences in the responses of callus (Tal et al., 1978; Orton, 1980) and protoplast (Rosen & Tal, 1981) cultures, and salt tolerance in halophytes appears to include a substantial cellular basis in some species (Hedenstrom & Breckle, 1974; Warren & Gould, 1982) but not in others (Smith & McComb, 1981).

Of particular interest here are results relating to a role of proline in relation to salt tolerance (Steward & Lee, 1974). Katz & Tal (1980) and Dix & Pearce (1981) have demonstrated elevated levels of proline synthesis in both salt sensitive and tolerant cell cultures in response to salt but exogenous proline was unable to ameliorate the effects of salt on a sensitive cell line of *N. sylvestris* (Dix et al., 1983). A protective effect of proline has, however, been observed in whole plants (Bar-Nun & Poljakoff-Mayber, 1977) and organised tissues (Mathur et al., 1980). These findings suggest a link between selection for salt tolerance and selection for amino acid overproduction via amino acid analogue resistance (Widholm, 1975). This link is supported by the recent results of Kueh & Bright (1982) who have demonstrated both proline accumulation and enhanced salt tolerance in hydroxyproline resistant barley plants.

We have been investigating the effect of exogenous proline on inhibition of *N. sylvestris* seedlings and callus by salt, and the characteristics of a hydroxyproline resistant line of *N. sylvestris*.

Materials and methods

Detailed conditions for the initiation and culture of *N. sylvestris* callus, and plant regeneration, have been described in earlier papers (Dix et al., 1977; Dix, 1981). Callus and seedling growth tests were performed as described by Dix et al. (1983).

The hydroxyproline resistant line, HPR105, was isolated from *N. sylvestris* (NS) callus culture on medium supplemented with 10 mM hydroxyproline. HPR105A is a callus line initiated from a regenerated plant and retaining its hydroxyproline resistance.

Amino acid extraction and analysis was performed as described previously (Dix & Pearce, 1981).

Results and discussion

The effects of proline on NS callus cultures were investigated at lower concentrations (proline at 0.1 and 0.5 mmol/l) than previously (Dix et al., 1983). Growth of NS callus is largely inhibited at 1% NaCl and completely at 1.5% NaCl. Neither of the proline concentrations tested gave any improvement of growth on saline medium and the higher concentration was itself partly inhibitory.

Using NS seedlings, however, a pronounced protective effect of 0.1 mmol/l proline was found at 1% NaCl (Table 1), as assessed by both shoot and root growth. The more severe stress caused by 1.5% NaCl could not be reversed, and at 0.5 mmol/l proline itself became strongly inhibitory.

These results suggest that exogenously applied proline can protect differentiated (seedlings) but not undifferentiated (callus) *N. sylvestris* against salt stress.

Amino acid analysis shows the hydroxyproline resistant (HPR105A) callus to contain 14.21 μmol proline g^{-1} fresh weight, as compared to 0.16 μmol g^{-1} in sensitive NS callus, representing almost 100× increase in free proline. In regenerating HPR105A tissue, consisting mostly of shoots, this falls again to 0.60 μmol g^{-1}, only four times higher than the sensitive control yet comparing favourably with the three times increase found by Kueh & Bright (1982) in the leaves of hydroxyproline resistant barley plants.

Preliminary investigations on the salt tolerance of HPR105A suggest a small increase in tolerance (compared to NS callus) of undifferentiated callus, and a more pronounced increase in the case of regenerating tissue, although in neither case does this compare with that found in undifferentiated lines selected directly for salt tolerance (Dix et al., 1983). Thus cell cultures obtained by this indirect selection procedure have a lower level of salt tolerance than those selected for growth in medium containing NaCl, but there may be a greater chance of the tolerance being expressed in the intact plant, and resulting from a stable genetic change.

Selection procedures of this kind based on careful consideration of physiological or biochemical mechanisms should also be applicable to other agronomic traits, including yield, and resistance to various stresses, such as those caused by temperature extremes, metal ions, herbicides and diseases.

Table 1. Effect of proline on salt tolerance of seedlings of *Nicotiana sylvestris*. Mean values of shoot fresh weight and adventitious root length of seedlings after 6 weeks on medium containing NaCl and/or proline.

Proline mmol/l	Shoot weight (g)		Adventitious root length (mm)	
	0% NaCl	1% NaCl	0% NaCl	1% NaCl
0	0.10	0.02	69	6
0.1	0.08	0.09	24	24

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